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Pasteurized milk quality in Brazil: a cross-sectional study over five years

Aline Martins Mancebo, Marina Rocha Dorella, Samanta Stinghen de Abreu, Stael Málaga Carrilho, Giulia Ferracin Ferreira, Rafael Mikio Itida, Willian Caixeta Gutierres Correia, Fernanda Montanholi de Lira, Ronaldo Tamanini and Rafael Fagnani

Laboratório de Inspeção de Produtos de Origem Animal – LIPOA, Department of Preventive Veterinary Medicine, Universidade Estadual de Londrina – UEL, Londrina, PR, Brasil

Research Article

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Author for correspondence:
Rafael Fagnani,
Email: rafaelfagnani@hotmail.com

Abstract

This research communication delineates the quality of pasteurized cow milk sold in Brazil from 2015 to 2020. A cross-sectional study was performed gathering 1749 samples, which were evaluated for microbiological and physicochemical parameters, including *Salmonella* spp., total and thermotolerant coliforms, freezing point, alkaline phosphatase and lactoperoxidase. The proportion of compliant and non-compliant samples was compared through the years and jurisdiction of the inspection services. Interactions between the design and response variables were assessed by log-linear analysis. Overall, a considerable non-conformity rate (12%) was found for at least one microbiological or physicochemical parameter. Post-pasteurization contamination by coliforms was the major challenge for dairy industries. Notably, the non-compliance rate for freezing point increased during the SARS-CoV-2 pandemic. In addition, the ability to comply was linked to the type of inspection service. Thus, it is suggested that the SARS-CoV-2 pandemic is affecting the dairy industries in Brazil, and we strengthen the need for more studies monitoring the quality of milk over the years, which could assist industries and regulatory agencies to ensure the compliance of pasteurized milk.

The volume of cow milk produced in Brazil has risen steadily over the last several years, hovering around 34 billion liters each year. In 2021, Brazil produced approximately 25 billion liters of milk for human consumption (IBGE, 2022). Pasteurized milk processing is very common among Brazilian dairy industries, present in about 1 in 4 dairy establishments under the Brazilian Federal Inspection Service (Lima *et al*., 2017). Microbiological spoilage is one of the main challenges in the pasteurized milk supply chain, which may occur at various points throughout the production system, from raw milk to post-pasteurization contamination. These microorganisms can grow at refrigerated temperatures and produce extracellular enzymes, resulting in off flavors, shelf-life shortage, and consequently dairy waste. Food loss is a global concern amnd it is estimated that as much as a quarter of dairy products are lost annually (Martin *et al*., 2021). Thus, studying the quality of pasteurized milk represents an opportunity for potential economic impact.

Specifically for pasteurized milk, the three main issues often described are microbiological post-pasteurization contamination through spoilage bacteria, aflatoxin M1 contamination and contamination by pathogenic bacteria and the search for virulence genes. The major microbiological contaminants are *Pseudomonas* spp., coliforms and Gram-positive spore-forming bacteria (Martin *et al*., 2018) and it is important to recognize that the pasteurization process cannot reduce the concentration of aflatoxins (Mollayusefian *et al*., 2021). The last research conducted in Brazil showed that 36% of pasteurized milk samples were positive for *Bacillus cereus* toxin genes (Reis *et al*., 2019). It is rare but not unknown for pasteurized milk to be linked to disease outbreaks: a recent systematic review of 31 studies conducted between 2007 and 2020 found three outbreak sources linked to pasteurized fluid milk consumption in Canada or USA, and *Listeria monocytogenes* was the most common pathogen involved (Sebastianski *et al*., 2022).

Besides microbiological analysis, the physicochemical composition of pasteurized milk is a crucial indicator of quality and safety. The freezing point is an internationally recognized method to detect milk adulteration by the addition of water. This parameter is also affected if acidity is developed and by addition of foreign substances to disguise the water addition, such as sodium chloride, ammonium sulfate, sucrose, phosphate and/or citrate salts. Determining the enzymatic activity of alkaline phosphatase and lactoperoxidase is useful for controlling the efficiency of milk pasteurization, ensuring that the product is safe for consumption (Nyokabi *et al*., 2021).

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The quality of pasteurized milk is often described in studies with sampling collection from retail sales, but most reports are restricted regarding the representativeness of the sample, often compounded by small sample sizes or selection bias that does not accurately reflect the target population. Thus, we emphasize the need for more studies monitoring the quality of milk over the years with proper sampling, the results of which can assist industries and regulatory agencies to ensure the compliance of pasteurized milk. Thus, considering the relevant role of pasteurized milk in the world dairy supply and the lack of studies monitoring its quality, our study aimed to investigate the microbiological contamination and physicochemical identity of pasteurized milk sold in the north and northwest State of Paraná, Brazil, from 2015 to 2020.

Materials and methods

Samples

A cross-sectional study was performed on data collected from 1749 pasteurized cow milk samples from January 2015 to December 2020. The sampling plan was part of the market surveillance conducted by the Brazilian Health Regulatory Agency (Anvisa). The sampling covered pasteurized milk from 21 dairy industries from 13 municipalities of the north and northwest Paraná State, Brazil. All samples were stored in an ice cooler filled with ice packs (<10°C) and immediately transported to the laboratory for microbiological and physicochemical analyses.

Analyses

The samples were analyzed for freezing point assessment by thermistor cryoscope (PZL 7000S, Londrina, Brazil) (ISO 5764:2009), for alkaline phosphatase (AOAC, 2016) and for lactoperoxidase (ISO, 2011). *Salmonella* spp. were determined following the recommendations of ISO 6785:2001. Total and thermotolerant coliforms were enumerated according to ISO 4831:2006 and ISO 7251:2005 methods, respectively.

Statistical analysis

The results from physicochemical and microbiological analysis were based on the percentage of samples that were compliant to the parameters set in the statutory Brazilian regulation for pasteurized milk. The proportion of compliant and non-compliant samples for each microbiological and physicochemical parameter were analyzed by Chi-Square through the years (2015–2020) and jurisdiction of the inspection services (federal, state, or municipal). In addition, the interactions between the design and response variables were assessed by Log-Linear Analysis for statistical significance. All analyses were performed in Statistica software 12.0 release, with statistical significanc ebeing declared at $P < 0.05$ or better.

Results and discussion

From 2015 to 2020, 12% of 1749 samples of pasteurized cow milk were in non-compliance for at least one microbiological or physicochemical parameter (Fig. 1). The most frequent non-conformity was failure to meet the total coliforms requirement. About 8% of samples were above the statutory limit of 4 MPN/ml (Fig. 1). The mean value and standard deviation for total coliforms was 4.18 (±18.10) MPN/ml, ranging from <0.3 to 111 MPN/ml.

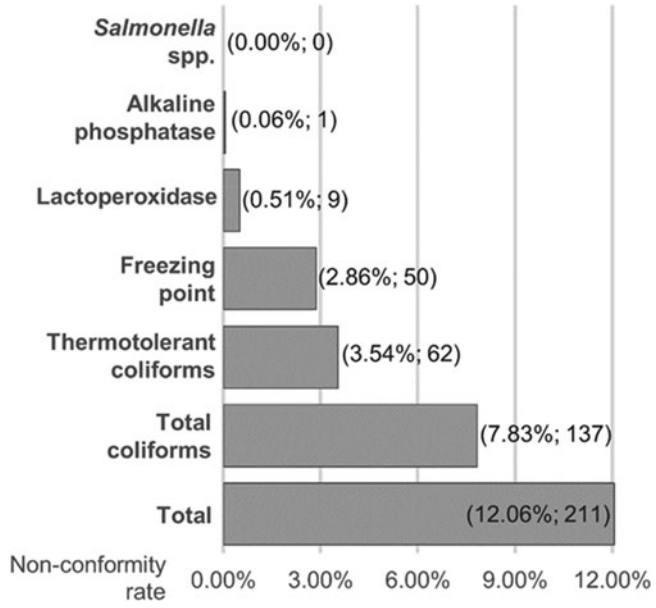


Fig. 1. Non-conformity rate of 1749 samples of pasteurized cow milk analyzed from 2015 to 2020 and categorized by microbiological and physicochemical parameters.

Based on these results, we can suggest that the biggest challenge for the dairy industries from the north and northwest Paraná State was to meet the microbiological criteria since 11% of the analyzed samples disagreed with the requirements of total or thermotolerant coliforms. Almost all samples (99.99%) contaminated by coliform bacteria were negative for alkaline phosphatase, indicating post-pasteurization contamination. Although coliforms represent less than 20% of the total post-pasteurization contamination, they can significantly reduce shelf life due to the production of proteases and lipases by many strains. Masiello *et al.* (2016) found that 71 and 43% of coliforms isolates displayed proteolysis or lipolysis, respectively. Even at refrigeration temperatures (5–6°C), the spoilage caused by coliforms can be detected sensorially within 7–10 d of processing (Martin *et al.*, 2018). Furthermore, most of coliforms are environmental contaminants, but a fraction of this group can also indicate fecal contamination, which represents poor hygienic conditions through milk processing in both cases (Martin *et al.*, 2016).

From our results, it is possible to infer that the coliforms post-pasteurization contamination is the major barrier for some dairy processors in the north and northwest Paraná State. The three main reasons for this kind of issue are flaws in cleaning and sanitization protocols, in cross-contamination control or from a lack of preventive maintenance. Thus, efforts are clearly needed to reduce all post-processing contaminations. The root cause analysis and the source tracking of contamination are good approaches to identifying the causative elements of post-pasteurization contamination. In addition, molecular tools such as pulsed-field electrophoresis, ribotyping, multilocus sequence typing and DNA-based techniques are useful to discriminate between persistent (ie biofilms) and transient contaminations (ie cross-contamination: Martin *et al.* 2018; Zoellner *et al.* 2018; Nakamura *et al.* 2021).

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Table 1. Non-conformity rate of microbiological and physicochemical parameters of 1749 samples of pasteurized cow milk categorized by year and jurisdiction of the inspection services (federal, state or municipal)

	Freezing point	A. phosphatase	L peroxidase	Total coliforms	Thermotolerant coliforms	*Salmonella* spp.
Year						
2015 ($n = 214$)	3.74%b	0.00%a	1.40%a	7.01%a	2.80%a	0.00%
2016 ($n = 310$)	0.00%a	0.00%a	0.32%a	9.03%a	3.23%a	0.00%
2017 ($n = 333$)	0.00%a	0.00%a	0.30%a	5.11%a	2.70%a	0.00%
2018 ($n = 380$)	0.00%a	0.00%a	0.53%a	7.89%a	2.89%a	0.00%
2019 ($n = 392$)	0.00%a	0.26%a	0.26%a	8.42%a	4.59%a	0.00%
2020 ($n = 120$)	35.00%c	0.00%a	0.83%a	11.67%a	6.67%a	0.00%
χ^2 P	488.64 <0.01	2.99* 0.70	3.68* 0.60	6.88 0.23	6.27 0.28	–
Inspection						
Fed. ($n = 405$)	1.25%a	0.00%a	0.25%a	6.23%a	1.75%b	0.00%
St. ($n = 1132$)	2.04%a	0.00%a	0.62%a	8.00%a	3.82%ab	0.00%
Mun. ($n = 212$)	10.63%b	0.48%a	0.48%a	9.18%a	5.80%a	0.00%
χ^2 P	50.97 <0.01	5.26* 0.15	0.92* 0.63	0.80 0.67	7.05 0.03	–

*G test.

The most frequent physicochemical non-conformity was the freezing point, with about 3% of the samples in disagreement (Fig. 1). Of these, 84% were above the country's statutory limit of −0.530°H, indicating water addition. This non-conformity can happen from deliberate addition of water, which is an economical food fraud, or from stagnant residual water caused by rinse failures or even problems with the clean-in-place (CIP) system design of the dairy industries (Ansari *et al.*, 2018). Thus, the Brazilian inspection services should drive efforts not only to combat deliberate water addition but also routinely audit the current CIP elements in dairy industries, which is crucial to ensure operational efficiency and pasteurized milk quality.

Furthermore, stagnant water is a significant source of microbiological contamination (Benyagoub *et al.*, 2018). Indeed, the log-linear analysis in our study showed that the non-conformity rate for coliforms was 2.1 times higher in samples with freezing point above −0.530°H (16.67%) compared to samples with regular freezing point (7.59%). The strength of the association between high freezing point and coliforms contamination had an odds ratio of 2.19, with confidence interval (95%) ranging from 1.09 to 4.39, and was statistically significant ($P < 0.05$).

We also observed an association between the non-conformity for freezing point and the jurisdiction of the inspection services (Table 1). In Brazil, the manufacturing of food from animal origin must be registered in one of the three levels of government administration (Federal, State or Municipality). The association observed in this study between the non-conformity for freezing point and the jurisdiction of the inspection services is argued by Carneiro and Kaneene (2017), who discussed that the inspection service at the Municipality level tends to be looser, associated with frequent changes to the organizational structure of the veterinary services, resulting in lack of sustainability of policies and a consequential problem with the uniform implementation of policies and actions.

The non-conformity rate of the freezing point was much higher in 2020, with 35% of the samples exceeding −0.530°H (Table 1). As stated above, the high freezing point of pasteurized milk could reflect either economically motivated adulteration or issues in milk processing technology, such as poor system design with low-drain areas and water stagnant conditions. This was the most frequent nonconformity observed in our study in 2020, with a marked difference when compared to previous years. Before considering the most likely cause for this non-conformity, it must be noted that in January 2020 the novel coronavirus outbreak was declared as a public health emergency of international concern by The World Health Organization. By mid-May 2020, Brazil had become the epicenter of the pandemic, reporting the second highest number of cases in the world (Neiva *et al.*, 2020). During the outbreak, morbidity and mortality increased and caused significant economic and social disruption, which also affected the dairy sector. Acosta *et al.* (2021) used a longitudinal qualitative analysis framework and concluded that the social distancing measures along with irregular access to processing inputs affected the productivity of dairy industries, which also have faced challenges from influx of employees and consequential losses in production efficiency and processing quality. Despite the lack of studies at national level about the milk supply chain during the pandemic in Brazil, we do know that the outbreak affected both the production and processing of animal products worldwide (Hashem *et al.*, 2020). This ranged from a shortage of farming inputs (such as animal feed, fuel, milking machines and vaccines) to problems with human resources in farms and dairy industries, including a lower than normal number of qualified workers and veterinary inspectors (Wolf *et al.*, 2021). By following this line of reasoning, it is fair to deduce that deliberate adulteration of milk (there is less liklihood of being caught) and processing deficiencies are more likely to occur. Cross-sectional studies do not necessary imply causal conclusions, however, it is suggested that the SARS-CoV-2 pandemic has affected the dairy industries in Brazil.

In conclusion, from 2015 to 2020 the biggest challenge for the dairy industries from the north and northwest Paraná State was to

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avoid the microbiological contamination of pasteurized cow milk. Furthermore, we suggest that the changes caused by SARS-CoV-2 pandemic through 2020 affected the ability of the dairy industries in meeting some requirements set in the statutory Brazilian regulations for pasteurized milk. Finally, our study highlights the need for better tools to minimize post-pasteurization contamination in dairy industries, as well as enhance the audit to improve fraud prevention and detection.

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